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United States
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Agriculture

**Agricultural
Research
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February 1993

Agricultural Research Service Progress Report

The Russian Wheat Aphid Fifth Annual Report

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PSWCL Prog. Rep. 93-009

Contents

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Introduction

It has been almost seven years since the detection of the Russian wheat aphid (RWA) in the United States, and the insect continues to thrive as an economic pest of wheat, barley, and certain forage grasses. The latest economic data[1] indicates that during the growing season of 1990-91, RWA infestations resulted in 276,100 insecticide-treated acres and an estimated 1.4 million bushel loss in grain production. Estimated cost of treatment was $2.5 million, and estimated value of grain loss was $3.5 million, for a combined loss of nearly $6 million. Estimates[1] of cumulative losses for 1987-91 are $331.2 million in direct loss (control costs, yield loss, and grazing loss) and $337.2 million in indirect loss--a total loss approaching $670 million.

Losses caused by RWA were lower in 1990-91 than in previous years, but it would be highly erroneous to conclude from these data that the RWA is becoming a less important North American small grain pest. RWA populations will undoubtedly fluctuate over the years with fluctuations in the environment. It is known that extended winter snow cover and wet spring snows as well as prolonged drought can have a major impact on RWA populations. Even though the RWA was first detected in 1978 in South Africa, it continues to be a devastating pest in that country, especially in the Orange Free State. We cannot assume the situation will be otherwise in North America. The RWA is now established over a very large part of North America, as pointed out by Brooks and Amosson,[1] and serious outbreaks will continue to occur when conditions are favorable. In fact, during the fall of 1992, infestations as high as 70-100% were found and treatments applied in a few isolated areas of western Nebraska. These infestations were as high as they had ever been in western Nebraska. In Colorado, fall RWA populations were high on the western slope in 1992. Also in 1992, the RWA reached its highest numbers since its detection in the Lethbridge, Canada, area in 1988, with very high numbers reported in the fall. Many fields of winter wheat sown in the last week of August and the first week of September were killed.

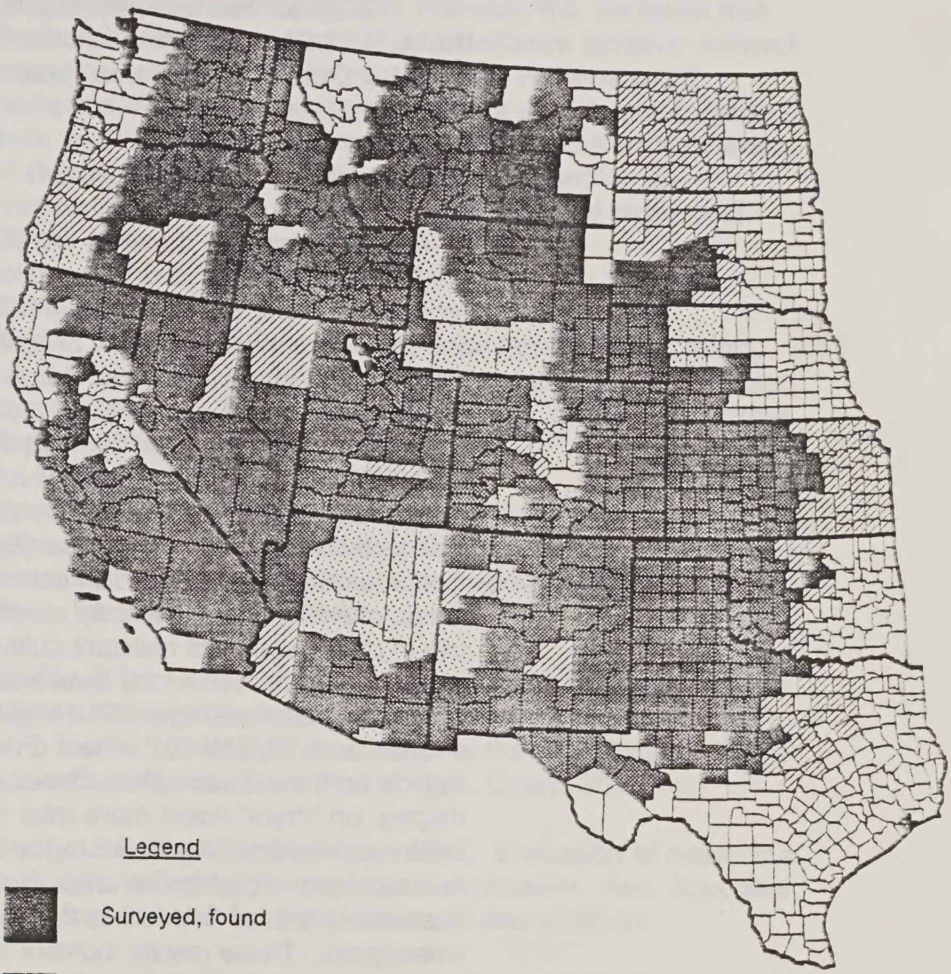
This is the fifth annual report of research progress on RWA by the Agricultural Research Service of the USDA. Previous reports were compiled by the late Robert L. Burton. Dr. Burton was in the process of compiling this report at the time of his death. Future reports will be compiled by James A. Webster of the Stillwater, OK, ARS Plant Science Research Laboratory. The report is intended as a brief update on the advances in technology that have taken place during the past year in this organization. However, many of these projects are cooperative with other organizations such as other ARS scientists and

[1]Brooks, L., and S. Amosson. 1993. Economic impact of the Russian wheat aphid in the western United States. Great Plains Agric. Counc. Pub. 143.

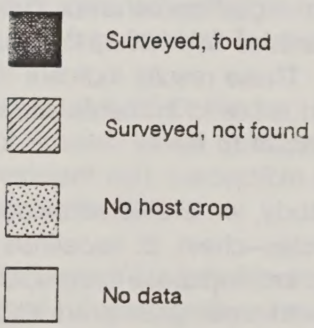
locations, state universities, experiment stations, and USDA-APHIS. These combined efforts toward solving the RWA problem have been remarkable, and greatly increase the possibility of successfully managing this pest in the future.

Space limitations for this report dictate brevity. Additional detail or information about the RWA or specific projects may be obtained directly from the researchers listed in the individual sections.

RUSSIAN WHEAT APHID, 1986-1992



Legend



Map by D. Cooksey
Montana State University
Department of Plant and Soil Science
November 7, 1991

Cooperative Agricultural Pest Survey (CAPS)
Map based on data from the National Agricultural Pest Information System (NAPIS)

Alternate Hosts for Russian Wheat Aphid

Mission: To identify and characterize RWA-resistant germplasm lines that may serve as breeding resources for both cool- and warm-season cereals and turf, range, and conservation grass species.

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RWA poses a serious threat to the yield and quality of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) production in the United States. Although Land Grant College and Soil Conservation Service programs advocate the use of many cool- and warm-season forage grasses, many of these grasses serve as alternate hosts to the RWA. One form of cultural control is the development of grass cultivars resistant to RWA for use in such programs, which could eliminate oversummering host plants for the aphid. Prior studies have indicated that slender wheatgrass (*Elymus trachycaulus* (Link) Gound ex Shinners), a species commonly found in reclamation areas, offers several potentially resistant plant introductions for use in development of resistant cultivars. The nature of RWA resistance at the behavioral level was studied with four slender wheatgrass entries--'Pryor', PI 387888, PI 440100, and PI 440102--and 'TAM W-101' wheat on electronic feeding monitors. Aphids on the resistant plant introductions and, to a lesser degree, on 'Pryor' spent more total time engaged in baseline behavior, less time in phloem-ingestion behavior, and more time in nonphloem-ingestion behavior than did aphids on the susceptible entries 'TAM W-101' wheat and PI 387888 slender wheatgrass. These results indicate that PI 440100 and PI 440102 could be used in the development of slender wheatgrass cultivars resistant to RWA.

In another study, we evaluated selections from three annual *Bromus* species--cheat, *B. secalinus* L.; downy brome, *B. tectorum* L.; and Japanese brome, *B. japonicus* Thunberg--associated with small-grain production and having life cycles similar to that of winter wheat, *Triticum aestivum* L. Objectives were to establish preferences of RWA for cheat, downy brome, Japanese brome, and wheat and to evaluate *Bromus* species indigenous to the central and western United States for their relative susceptibility to aphid feeding damage. Studies of nonpreference, aphid weight and fecundity, time to plant death, and plant damage were conducted in a greenhouse. The following order of decreasing acceptability was observed: wheat, downy brome, cheat, and Japanese brome. These data suggest that RWA prefer wheat in a wheat-annual brome mixture. RWA reared on wheat were on average 38% heavier and produced an

average of 10.2 more nymphs per individual than when reared on annual brome species. Seedlings of wheat infested by RWA lived 6.7 days less than the brome species.

In a separate study using plant introductions of several *Bromus* spp. obtained from the USDA/ARS Plant Germplasm Introduction and Testing Station, Pullman, WA, we found that *Bromus inermis* Leysser and *B. pumpellianus* Scribner suffered minor feeding damage. *Bromus erectus* Hudson and *B. biebersteinii* Roemer & Schultes sustained moderate damage from feeding aphids, and 14 other species received high levels of damage. Field studies are needed to determine the relationships of native plant communities, cereal crops, and RWA.

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Host Plant Resistance

Mission: To identify resistance sources, study the nature of this resistance, and cooperate with the Small Grain Germplasm Enhancement program in the development and release of RWA-resistant small grain germplasms.

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Germplasm Evaluation

Evaluation of plant germplasm for pest resistance is a basic component of all host plant resistance programs A systematic germplasm evaluation program was initated in Stillwater soon after the detection of the RWA in 1986. As of this writing, over 43,000 small grain lines from the USDA-ARS National Small Grain Collection in Aberdeen, ID, have been evaluated at least once for resistance to this pest. Evaluations have been conducted with seedling-stage plants in the greenhouse. Previous experience has shown that aphid-resistant seedlings are almost always resistant in later plant growth stages. Results of these tests have been documented in 17 reports that have been sent to the Curator of the National Collection, where the information has been entered in the GRIN (Germplasm Resource Information Network) system. Public and private plant breeders can access RWA information on any line tested in Stillwater from National Small Grain Collection records via the GRIN system. State agricultural experiment station, commercial, and federal plant breeders are now using many of the RWA-resistant lines first detected in Stillwater in their breeding programs, with the goal of developing RWA-resistant germplasm and cultivars for the American small grain industry. Plant breeders in other countries such as South Africa and Mexico are also very interested in these lines.

Since this is the Fifth Annual RWA Report, a breakdown of lines tested from the ARS National Collection seems appropriate.

> <u>Barley</u> - Number of lines tested - 21,822
> We have essentially completed the mass evaluation of this collection. At least 32 lines were found to have good resistance. We are currently testing 927 lines that were previously unavailable, or unclassified, or are new introductions.
>
> <u>Wheat</u> - Number of lines tested - 19,655
> Over one third of this collection has been tested. Current plans are to complete tests of this collection during the winter of 1993-94. A recent paper accepted

by the scientific journal Plant Breeding lists 29 RWA-resistant lines discovered by ARS in Stillwater. The paper was coauthored by scientists in the Germplasm Enhancement and Host Plant Resistance programs, and will be published in 1993.

Rye - Number of lines tested - 1,238
All available lines were tested in 1989. New introductions and lines previously unavailable will be tested in the future. There is considerable RWA resistance in this collection. Nine lines were saved for additional evaluations. With the discovery of RWA resistance in common wheat lines, use of these rye lines in wheat breeding programs has dwindled in the interest of the rapid development of RWA-resistant germplasm. However, should RWA biotypes occur, these rye lines may prove to be valuable resistance sources in the future.

Triticale - Number of lines tested - 731
Triticale is a relatively new crop that is produced by crossing wheat and rye. All available lines were tested in 1987. Seven resistant lines were found in laboratory tests and reported by Webster in 1990. In the summer of 1992, Dr. Harold Bockelman, Curator of the National Small Grain Collection, reported that four of these accessions--PI 386148, 386149, 386150, and 386156--exhibited high levels of resistance in an Aberdeen, ID, field nursery severely infested with RWA. As with the rye germplasm, new introductions and lines previously unavailable will be tested in the future.

In addition to the accession from the National Collection, a large number of advanced breeding lines have been tested for RWA resistance in cooperation with the Stillwater ARS Germplasm Enhancement program and other plant breeders. We have been quite successful in detecting RWA-resistant germplasm using current technology; however, we are continually striving to develop new evaluation methods to make the process more efficient. Beginning with the 1992-93 tests, we are using a new RWA colony originating from equal proportions of RWA collected from Colorado, Oregon, and Idaho earlier in the year. This colony is more representative of current RWA field populations. We still maintain a small colony of the 1986 Texas RWA. New metal halide lights have been installed in the testing greenhouse to extend the daylength to 14 hours and to provide supplemental light on cloudy days. These lights will greatly improve plant growing conditions during winter months. A new seed storage controlled-temperature room was put into operation in 1992. This will enhance our capabilities to preserve valuable germplasm lines for future research.

Nature of RWA Resistance in Small Grains

As mentioned in the last report, studies with the electronic feeding monitor show that RWA confined to a resistant barley line (PI 366450) spend less time in phloem feeding activities than do RWA on susceptible lines. These results will be published in the Journal of Economic Entomology in 1993. Data are now being analyzed on RWA feeding behavior on different growth stages of resistant and susceptible barley. The computerization of the electronic feeding monitor system is nearly completed. In addition to saving large amounts of recording paper, the new system will be much more versatile. Feeding patterns can be studied in more detail, and many new types of experiments can be conducted. A new technique for measuring aphid host preference with great precision was developed.

Field Tests

A tritrophic field test was conducted in cooperation with the Stillwater Biological Control team during 1991-92. The test is being repeated this year. We cooperated with four other U.S. locations in the First Uniform Russian Wheat Aphid Field Test. The four Stillwater ARS lines in the test exhibited high levels of RWA resistance at Stillwater.

Other

A cooperative test conducted in South Africa and Stillwater shows that an RWA colony originally collected in Texas and reared on barley in the greenhouse for over two years does not reproduce well at a constant 25°C. The results are reported in a publication accepted by the Journal of Economic Entomology. Additional research about temperature effects on the RWA is planned.

International Cooperation

A strong network of international cooperation in the host plant resistance and germplasm enhancement area has been developed. Three South African scientists and a CIMMYT (Mexico) wheat breeder visited these programs during 1991-92. A Stillwater scientist conferred with scientists at both of these locations during 1990-91. A one-week training session in RWA host plant resistance techniques was provided for a student from The Sudan. A group of wheat lines from Assuit University (Egypt) was tested for RWA resistance.

Publications Since Last Report

Baker, C.A., J.A. Webster, and D.R. Porter. 1992. Mechanisms of Russian wheat aphid resistance: Identification of antibiosis in hexaploid wheat, pp. 90-93. *In* W.P. Morrision (comp.) Proceedings of the Fifth Russian Wheat Aphid Conference. Great Plains Agric. Counc. Pub. 142.

Porter, D.R., J.A. Webster, and C.A. Baker. Detection of resistance to the Russian wheat aphid in wheat. Plant Breeding (In press)

Webster, J.A. 1992. Host plant resistance, pp. 10-11. *In* R.L. Burton (comp.) The Russian wheat aphid, fourth annual report. U.S. Dep. Agric., Agric. Res. Serv. PSWCL Prog. Rep. 92-001.

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Webster, J.A., D.R. Porter, C.A. Baker, and D.W. Mornhinweg. Russian wheat aphid (Homoptera: Aphididae) resistance in barley: Effect on aphid feeding. J. Econ. Entomol. (In press)

Small Grain Germplasm Enhancement

Mission: To identify, characterize, and introgress genes conferring RWA resistance for small grain germplasm enhancement.

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Wheat

Currently, wheat germplasm enhancement is concentrating on a core collection of 29 RWA-resistant lines. Each selection has been hybridized with adapted RWA-susceptible wheat cultivars; F_1, BC, F_2, and F_3 generations have been produced. All generations will be used to determine the inheritance of RWA resistance in each of the 29 selections. To date, the genetics of RWA resistance has been determined in 2 of the 29 lines; PI 140207 has a single dominant gene for resistance, and in PI 149898 resistance is controlled by two genes. An accurate determination of the number of genes in PI 149898 required an analysis of segregation ratios in the F_3 generation. Based on F_1, BC, and F_2 data alone it would have been easy to classify F_2 segregation patterns into a simple 3:1 ratio. It does require extra time and effort to look at advanced generations, but in cases where classifications are not always clear (e.g., where is it accurate to draw the line between resistance and susceptibility on a scale of 1 to 9?), the increased level of certainty is well worth the extra time. Determination of the inheritance of resistance in several other lines is ongoing.

Genetic analyses are planned to determine if these 29 selections carry different genes for RWA resistance. Crosses were made between as many lines as possible, and F_2 populations have been produced. Several of these populations will be screened this winter. Genetic analyses with other RWA-resistant lines (PIs 372129, 294994, 262660, 137739) are also planned.

Release of two RWA-resistant germplasms is anticipated for early 1993. These releases were developed from crosses between 'Bobwhite' and PI 149898. They are hard red semidwarf wheats with moderate resistance in the greenhouse and strong resistance in the field. Field tests have shown lush fall growth even with RWA infestation.

Cooperative work included RWA screening of wheat breeding lines from the Oklahoma State University wheat breeding program.

The transfer of RWA resistance from triticale accessions to wheat is onging. A resistant F_2 plant was recovered from a hybrid resulting from the cross of a highly RWA-resistant triticale, PI 386149, with 'Chinese Spring'. This plant was crossed with 'Chisholm' and 'TAM W-101', and resistant F_1 plants were recovered along with resistant F_3 plants. X-ray irradiated pollen of another highly resistant triticale, PI 386156, was used to pollinate 'Pavon'. F_1 plants were crossed with 'Pavon' (both ways), 'TAM-107', and 'TAM-200'. Resistant F_1, F_2, and BC plants were recovered and are being advanced.

Barley

Fifty-six barley lines, previously identified as RWA resistant, were grown in Fort Collins, CO, and Idaho Falls, ID, in the spring of 1992. These lines were evaluated in Fort Collins for agronomic traits and at both locations for malting quality. A subset of 11 lines was also grown in Laramie, WY, in a replicated field trial under natural infestation of RWA. The objectives of this study were to determine the persistence of RWA resistance throughout the life of the plant and to determine the level of seedling resistance in the greenhouse necessary for field resistance. A similar study was conducted in the greenhouse with the same 11 lines where plants were infested at three different growth stages and grown to maturity under constant RWA pressure. RWA populations increased astronomically due to the protective environment of the greenhouse. Under both conditions, field and greenhouse, RWA seedling resistance persisted throughout the life of the plant, and those lines with the highest level of seedling resistance performed the best at the whole-plant level.

Fifty-five successful crosses were made between 46 resistant lines and several malting barley cultivars. Seventy-two successful backcrosses were made, and 34 F_2 populations increased for use in future genetic studies. Seed was increased on 300 F_2 plants from the cross 'Morex'/STARS-9301B to obtain the F_3 families necessary for complete genetic analysis prior to the release of STARS-9301B as a RWA-resistant line. Nine hundred STARS-9301B plants were rescreened and bulked for release. Parent, F_1, F_2, BC, and F_3 populations of the remaining 80 lines previously identified as resistant will be evaluated in the coming years to determine genetic diversity for RWA resistance in barley.

Genetic studies included evaluation of backcross populations from 'Morex'/STARS-9301B. These data supported the results of last year's F_2 study that RWA resistance in STARS-9301B is under multiple gene control. The parental, F_1, F_2, and BC material is currently being evaluated again in one genetic test to establish repeatability of results. This information along with segregation ratios of 300 F_3 families with this cross should determine the inheritance of RWA resistance in STARS-9301B. Several genetic studies were conducted to determine if STARS-9301B carries the same genes for resistance as six other RWA-

resistant lines endemic to the same geographical area. Results indicate similar gene control for RWA resistance in these lines.

Cooperative efforts included screening 4,500 BC_1F_3 seedlings from wide crosses between *Hordeum vulgare* and *H. bulbosum* for USDA barley breeders in Idaho. Segregation was observed in 360 F_3 families for Busch Agriculture Resources Inc. and homozygous resistant lines identified. Eleven advanced populations from wide-cross hybridization of *H. vulgare* and *H. bulbosum* were screened for R.A. Pickering in New Zealand.

Cellular Resistance Studies

Efforts to characterize responses to RWA attack in wheat and barley leaf tissue continued. Protein synthesis and accumulation patterns in RWA-resistant and -susceptible barley genotypes before and after attack were described in last year's report. The same approach was taken to look at the effect of RWA feeding on resistant and susceptible wheat germplasm. Protein profiles of RWA-infested and noninfested leaf tissue of PI 140207 (RWA-resistant) and 'Pavon' (RWA-susceptible) were examined by visualizing silver-stained denatured proteins separated by two-dimensional polyacrylamide gel electrophoresis. Ten RWAs were confined in small clip cages for four days attached to the middle of the first leaf of 1.5-leaf-stage green seedlings. Leaf segments were taken from within the clip cage (direct RWA feeding sites) and from the leaf outside the cage (where no RWAs had been in contact with the leaf). Comparisons were made among the following treatments: 'Pavon' noninfested, PI 140207 noninfested, 'Pavon' infested - inside clip cage, 'Pavon' infested - outside clip cage, PI 140207 infested - inside clip cage, PI 140207 infested - outside clip cage. In general, very few differences were detected between 'Pavon' and PI 140207 in noninfested leaf tissue. Profiles of proteins taken from leaf sections outside the clip cages on infested plants showed dramatic differences. The protein profile of PI 140207 appeared identical to the noninfested PI 140207 tissue. On the other hand, 'Pavon' exhibited a dramatic decrease in the accumulation of a specific complex of proteins approximately 24 kD in weight. This differential response is virtually identical to that observed in the analysis of resistant versus susceptible barley genotypes (reported here last year). However, when leaf tissue of both genotypes (PI 140207 and 'Pavon') was exposed to direct feeding pressure for 4 days inside the clip cage, protein profiles of both genotypes showed similar selective inhibition of accumulation of the 24 kD protein complex. These differential responses are intriguing and lead to a series of interesting questions and lines of research. It does appear, however, that the cellular damage response of susceptible genotypes is the same in wheat as it is in barley.

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RWA-Host Plant Interaction

Mission: To develop a fundamental understanding of the molecular nature of the physiological and biochemical basis of RWA damage to facilitate the development of resistant germplasm derived from both traditional breeding programs and genetic engineering techniques.

Research in support of this mission is broadbased and involves studies both in the laboratory and greenhouse at Stillwater, OK, and in large-scale field plots located at Brookings, SD. Because each unit is studying a different aspect of the interaction phenomena, we have reported their progress separately to assist interested readers who may wish to seek additional specific information directly from the scientists involved.

Unit Mission: To characterize plant physiological and biochemical responses to RWA attack in resistant and susceptible germplasm to identify superior metabolic systems, pathways, or individual components critical to genetic resistance.

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An understanding of the host's response to pest attack is crucial in developing effective resistance evaluation protocols for germplasm enhancement efforts. Analysis of plant responses to RWA feeding included: 1) the production of ethylene (a plant wound hormone), 2) identification of collapsed, autofluorescent cells, and 3) photosystem stability and recovery characterized through the kinetics of chlorophyll fluorescence.

Levels of ethylene often rise in response to plant damage from wounding and other stresses. The role of ethylene in the barley/RWA interactions was investigated with two questions in mind: 1) does an imposed, elevated level of ethylene cause a plant response similar to an RWA infestation, and 2) does RWA feeding induce ethylene production in barley? To address the first question, barley plants were incubated in ethylene for 2 days and then compared with nonincubated plants after 2 weeks of growth. Other than shorter leaves, the ethylene-treated plants did not show damage responses characteristic of RWA feeding. To measure ethylene production during RWA feeding, plants were confined to closed test tubes and ethylene levels were measured after 150 or 300 aphids were added to each tube and allowed to feed. Under these test conditions, ethylene production was very low and variable. Similar results were found when using greenbugs instead of RWAs.

The analysis of leaf cellular responses from RWA feeding revealed a dramatic difference in the number of collapsed, autofluorescent cells in resistant and susceptible barleys. After 3 days of feeding, decolorized leaf sections where RWA fed were viewed with an ultraviolet light microscope. Resistant barleys had significantly higher numbers of fluoresceing cells than susceptible barleys. Fluorescence under these test conditions indicate that the cells contain a lignin-like substance. This discovery opens a new, exciting avenue for further research in plant responses associated with resistance.

Studies measuring transient changes of chlorophyll *a* fluorescence quantum yield in PS II-core complexes induced by RWA feeding stress indicate that an early event in the damage response involves the inhibition of Q_A-reoxidation. Photoinhibition, characterized by changes in F_o, F_M, F_V, F_V/F_M, and F_S, occurs during the first 2 hours of RWA feeding. Observed photooxidative injury (cell bleaching) in response to RWA feeding may involve the formation of activated oxygen species that arise from the disruption of the photosynthetic electron transport system which further react with chloroplast macromolecules causing their degradation. Current research is focused on evaluating the effect of RWAs on the turnover of D1, the 32 kDa herbicide-binding protein of PS II, and the functional integrity of cyclic carotenoids.

Publications Since Last Report:

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Burd, J.D., R.L. Burton, and J.A. Webster. Evaluation of Russian wheat aphid damage on different host species and comparisons of damage ratings with quantitative plant measurements. J. Econ. Entomol. (In press)

Burd, J.D., and G.W. Todd. 1992. Total chlorophyll and chlorophyll fluorescence profiles of Russian wheat aphid resistant and susceptible wheat, pp. 101-106. *In* W.P. Morrison (comp.) Proceeding of the Fifth Russian Wheat Aphid Conference. Great Plains Agric. Counc. Pub. 142.

Unit Mission: To study the effect of cropping systems on population dynamics of RWA, and determine how cultural practices affect plant stress and efficiency under RWA infestation.

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Laboratory research has indicated that grain yield loss to RWA in wheat (*Triticum aestivum* L.) can be reduced by increased levels of nitrogen fertilizer. Field application of nitrogen fertilizer, therefore, might be a useful strategy for limiting grain yield loss caused by RWA. To test this hypothesis, a field experiment with nitrogen fertilizer as main plot treatments and RWA infestation as subplot treatments was conducted. Soil tests from an established winter wheat field in early spring indicated that 78 kg/hectare nitrogen was needed to attain a potential grain yield of 4,800 kg/hectare. Nitrogen (liquid urea-ammonium nitrate) was then sprayed onto plants at rates of 0, 39, or 78 kg/hectare. Plants were infested with RWA for a period of 17 days. Plants given nitrogen fertilizer had increased plant dry weight, tiller number, leaf area, leaf chlorophyll content, and grain yield compared with plants given no nitrogen fertilizer. RWA infestation reduced grain yield approximately 31%. A lack of significant interaction between nitrogen fertilizer treatment and RWA infestation suggests that aphid infestation affected grain yield similarly for all fertilizer treatments studied. These results indicate that application of nitrogen fertilizer to winter wheat improves grain yield performance independently of the effects of RWA infestation.

Publications Since Last Report

Kieckhefer, R.W., and J.L. Gellner. 1992. Yield losses in winter wheat caused by low-density cereal aphid populations. Agron. J. 84:180-183.

Riedell, W.E., and R.W. Kieckhefer. 1992. RWA-host plant interaction: Northern Grain Insects Research Laboratory, p. 15. *In* R.L. Burton (comp.) The Russian wheat aphid, fourth annual report. U.S. Dep. Agric., Agric. Res. Serv., PSWCL Prog. Rep. 92-001.

Riedell, W.E., and R.W. Kieckhefer. Nitrogen fertilizer management and grain yield loss to Russian wheat aphids. Cereal Res. Commun. (In press)

Insect Genetics

Mission: To conduct national and worldwide biotypic and genetic studies on the RWA and its parasitoids.

In last year's report we noted that Dr. Gary Puterka who had been working in this research area at Stillwater, OK, as a postdoctoral Research Associate had accepted a position at the USDA-ARS laboratory at Kearneysville, WV. Additionally, during the period since the last report, Dr. Richard Roehrdanz at Fargo, ND, has completed his genetic studies of the RWA. Documentation of their research results in scientific journals continues, as shown below.

Publications Since Last Report

Black, W.C. IV, N.M. DuTeau, G.J. Puterka, J.R. Nechols, and J.M. Pettorini. 1992. Use of random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to detect DNA polymorphisms in aphids. Bull. Entomol. Res. 82:151-159.

Puterka, G.J., W.C. Black, IV, W.M. Steiner, and R.L. Burton. Genetic variation and phylogenetic relationships among worldwide collections of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), inferred from allozyme and RAPD-PCR markers. Heredity. (In press)

Puterka, G.J., and J.D. Burd. 1992. Insect genetics: Plant Science Research Laboratory, pp. 16-18. *In* R.L. Burton (comp.) The Russian wheat aphid, fourth annual report. U.S. Dep. Agric., Agric. Res. Serv., PSWCL Prog. Rep. 92-001.

Puterka, G.J., J.D. Burd, and R.L. Burton. 1992. Biotypic variation in a worldwide collection of Russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. 85:1497-1506.

Roehrdanz, R.L. 1992. Application of PCR techniques for identification of parasites and predators, pp. 190-196. *In* W.P. Morrison (comp.) Proceedings of the Fifth Russian Wheat Aphid Conference. Great Plains Agric. Counc. Pub. 142.

Roehrdanz, R.L. 1992. Insect genetics: Biosciences Research Laboratory, pp. 18-19. *In* R.L. Burton (comp.) The Russian wheat aphid, fourth annual report. U.S. Dep. Agric., Agric. Res. Serv., PSWCL Prog. Rep. 92-001.

Biosystematics

Mission: To provide identifications and verifications for RWA and its natural enemies.

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During FY92, M.B. Stoetzel continued to provide identifications and verifications for *Diuraphis noxia* (Mordvilko). There is a continuing need for voucher specimens to be submitted to the SEL as researchers search for parasites and predators of the RWA.

Dr. Stoetzel and R.W. Hammon (Fruita Research Center, Grand Junction, CO) have been collecting data on the biological development of species of *Diuraphis* with special emphasis on the occurrence of sexuales. Some of their findings were presented at the annual meeting of the Entomological Society of Washington.

From June 28 to July 4, 1992, Dr. Stoetzel attended the XIX International Congress of Entomology in Beijing, China. During the Special Interest Group Session "The Russian Wheat Aphid, *Diuraphis noxia* Mordvilko," Dr. Stoetzel gave an invited talk on "Taxonomy of the Russian Wheat Aphid and Related Species."

From July 6 to 9, 1992, Dr. Stoetzel worked with Prof. Zhang Guang-xue (Institute of Zoology, Academia Sinica, Beijing, China) and studied China-collected specimens of *Diuraphis noxia* (Mordvilko) and specimens of *D. agropyronophaga* and *D. elymophila*, two new species described in 1991 by Prof. Zhang. These species will be included in Dr. Stoetzel's taxonomic study of the genus *Diuraphis* now expected to be completed during FY93.

Publications Since Last Report

Stoetzel, M.B. 1990. The North American species in and related to *Diuraphis* Aizenberg. Acta Phytopathol. Entomol. Hung. 25:229-234.

Stoetzel, M.B. 1992. Biosystematics, p. 20. *In* R.L. Burton (comp.) The Russian wheat aphid, fourth annual report. U.S. Dep. Agric., Agric. Res. Serv., PSWCL Prog. Rep. 92-001.

Stoetzel, M.B. 1992. Taxonomy of the Russian wheat aphid and related species. Proc. 19th Int. Congr. Entomol., 28 June-4 July 1992, Beijing, China, p. 13.

Stoetzel, M.B., and R.W. Hammon. 1992. New collections of sexuales of *Diuraphis* (Homoptera: Aphididae) in North America. Proc. Entomol. Soc. Wash. 94:598-599.

Biological Control

Mission: To develop a fundamental understanding of the biological and ecological relationships between cereal aphids and their natural enemies in the agroecosystem landscape to facilitate the establishment and integration of biological control strategies into sustainable integrated pest management systems.

This multifaceted mission involves scientific expertise from several laboratories. Each unit's specific mission and accomplishments toward that mission will be reported as separate subsections.

Unit Mission: To develop strategies in the laboratory, greenhouse, and field for maximum utilization of natural enemies (exotic, naturalized, and endemic) in RWA-infested cereals and grasses.

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Goal: ***Develop principles for use in establishing and managing RWA parasitoids and predators in ephemeral agroecosystems in portions of the Great Plains.***

Scientists Involved: N.C. Elliott, D.K. Reed, J.D. Burd, G.J. Michels, Jr. (Texas A&M Univ.), G.L. Hein (Univ. of Nebraska), S.D. Kindler, B.W. French, C.A. Walker (Colorado State Univ.), F.B. Peairs (Colorado State Univ.)

Objectives

1) Determine the seasonal phenology and abundance of aphid species and their natural enemies in crops adapted to the Southern and Central Plains regions and in associated non-agricultural plant communities.
2) Develop methods for mass-marking aphid parasitoids to study foraging and dispersal in the field.
3) Determine the suitability of various aphid species common to crop and non-crop ecosystems in the Southern Great Plains as hosts to species/"strains" of RWA parasitoids.
4) Develop natural enemy release methods, and release natural enemies for the purpose of achieving colonization and establishment.
5) Evaluate the effectiveness of natural enemies in biological control of the RWA.
6) Determine the appropriate ecological scale for management of the RWA and its natural enemies in the ephemeral cropping systems that characterize the Southern and Central Great Plains.

For two successive years we studied the seasonal occurrence and abundance of aphid species in alfalfa, canola, sorghum, red clover, cotton, millet, cultivated sunflower, and vetch, and native grasses, as well as on several plant species common to uncultivated lands in the Southern and Central Plains. We identified 16 aphid species whose seasonal abundance patterns

indicate that they might effectively serve as alternate host/prey "bridges" for aphidophagous predators and parasitoids for use at times when RWA are typically absent from the agricultural landscape or present in extremely low numbers.

In laboratory tests we found that *Diaeretiella rapae* and *Aphidius colemani* imported for classical biological control of the RWA parasitized individuals of several of the comon aphid species to which they were exposed for 24 hours on caged host plants. Our results indicate that several of the 16 aphid species that commonly occur in wheat, in crops often grown adjacent to wheat, or in adjacent non-agricultural lands may serve as alternate hosts of these parasitoids.

Our results indicate that it may be possible to increase probabilities of establishment of RWA natural enemies by conducting releases at locations where adjacent habitats supporting alternate hosts occur. Results also provide knowledge of hosts and associated habitats into which RWA parasitoids might be released to attempt establishment at times when the RWA is temporarily absent in a particular geographic area. They also indicate a potentially important role for habitat diversification to improve biological control of the RWA (and other economically important cereal aphids) by increasing the availability and predictability of alternate hosts. Our results provide no evidence to suggest that inability to rapidly adapt to new hosts will limit the value of habitat diversification as an approach for increasing the effectiveness of these parasitoids in biological control of the RWA in the Great Plains. We are currently conducting similar studies with several geographic isolates of *Aphelinus asychis* and *A. sp. nr. varipes* from Asia.

A system has been successfully devised for the incorporation of radio-labeled markers into Russian wheat aphids, greenbugs, and their natural enemies. This technique has been adapted for use in both field and greenhouse research and studies are currently underway to determine RWA natural enemy disperal patterns, host selection, and efficacies (including competition) in multitrophic systems.

Caged releases consistently resulted in better colonization (greater natural enemy population densities) of release sites than open-field releases. In southeastern Colorado in 1992, caged releases in wheat fields resulted in greater population densities of natural enemies in colonization surveys than releases into Conservation Reserve Program (CRP) acreage. Follow-up surveys to determine whether establishment has occurred will begin in 1993.

In 1993 we will begin evaluation of RWA biological control in the Texas Panhandle in conjunctions with G. Michels and R. Deerberg (Texas A&M Univ.). We will determine the ability of the recently established *A. asychis* (and other natural enemies)

to suppress RWA populations in the field, and determine the impact of *A. asychis* on native parasitoids associated with small grains.

We are in the process of expanding our capabilities for quantitative study by establishing a quantitative laboratory with Geographic Information System (ERDAS) and Artificial Intelligence capabilities. These capabilities, when combined with existing capabilities for simulation modeling (including spatial simulation models), will facilitate rapid progress toward completion of objective 6.

Goal: ***Integrate host plant resistance with natural enemies in control programs for the RWA.***

Scientists Involved: D.K. Reed, J.D. Burd, R.K. Campbell (Oklahoma State Univ.), S.D. Kindler, R.D. Eikenbary (Oklahoma State Univ.), H.C. Reed (Oral Roberts Univ.), N.C. Elliott

Objectives

1) Determine the effect of different plant resistance mechanisms as mediated through the host on the population dynamics and biology of RWA parasitoids.
2) Identify the physical characteristics and biochemical factors associated with plant-aphid-parasitoid interactions and their relationship to parasitoid foraging behavior, host acceptance, and establishment.

Research on interactions among cereal hosts, RWA, and natural enemies is continuing. Research on interactions of entomophagous fungi, resistant and susceptible wheat, and RWA is in the final stages. Tritrophic effects on wheat grasses has been published, and work on long-term tritrophic effects and field tritrophic research is continuing.

As a visiting scientist from Oral Roberts University, Dr. H.C. Reed conducted research on olfactory cues to a common RWA parasitoid, *Diaeretiella rapae*, using a wind tunnel and olfactometer. His work has provided a great deal of preliminary information that will lead to further research this year. An in-house laboratory report and two student reports have been written concerning the research.

Goal: ***Elucidate details of basic laboratory and field biology of RWA natural enemies as an aid to assessing their potential for establishment and effectiveness against the RWA.***

Scientists Involved: N.C. Elliott, D.K. Reed, J.D. Burd, S.D. Kindler, G.J. Michels, Jr. (Texas A&M Univ.), B.W. French

Objectives

1) Obtain basic biological information on native and exotic RWA natural enemies.

2) Determine the effect of abiotic environmental variables on the potential for survival and population growth of RWA natural enemies.

We determined thermal thresholds for development of exotic *Aphidius colemani* and *Diaeretiella rapae*. Lower thermal thresholds are similar to that of the RWA, suggesting that low temperatures may not impose a barrier to population growth by these parasitoids.

We assessed the ability of *Cycloneda ancoralis* to successfully complete development on four aphid species (*Aphis gossypii, A. helianthi, Diuraphis noxia*, and *Lypaphis erysimis*) common in Great Plains agroecosystems. Results indicate that the coccinellid can complete immature development on all four species, but the species differ qualitatively as a food source for the coccinellid. While the ability to survive on a broad range of prey is insufficient to ensure that the coccinellid can establish and contribute to biological control of *D. noxia* and other cereal aphids, it is a desirable characteristic for successful exploitation of this agroecosystem.

Mummy weight, an easily obtained biological parameter often used as a measure of parasitoid robustness/fitness, was shown to be unreliable due to variance among growth stages. This variance can be eliminated with proper experimental protocols.

Field overwintering studies conducted in Oklahoma in 1990 and 1991 indicate that Soviet and Syrian *Diaeretiella rapae*, South American *Aphelinus asychis* and *Aphidius colemani*, and South American *Cycloneda ancoralis* and *Hippodamia variegata* can overwinter successfully in some winters. These studies are continuing.

Goal: ***Develop improved pest management systems for the RWA and other economically important aphid pests of small grains.***

Scientists Involved: N.C. Elliott, G.J. Michels, Jr. (Texas A&M Univ.), G.L. Hein (Univ. of Nebraska), R.W. Kieckhefer (ARS, Brookings), R.L. Deerberg (Texas A&M Univ.), J.D. Burd, D.K. Reed, S.D. Kindler

Objectives

1) Construct and validate computer simulation models for the RWA and other cereal aphids.
2) Develop improved field monitoring methods for RWA, other cereal aphids, and natural enemies.
3) Determine economic thresholds for economically important aphid pests of small grains.

We found that removal sampling provided acceptable estimates of the absolute population density of adult coccinellids in wheat

fields. Estimates of absolute density, obtained by removal sampling, were used to convert estimates of relative population density obtained from visual counts taken while walking at a constant velocity, to estimates of absolute population density. A sequential sampling scheme for estimating the mean number of adult coccinellids per two-minute count with known average precision was developed.

Removal sampling provides accurate estimates of larval coccinellid populations at low and intermediate densities, but at high densities the efficiency of removal sampling is too low to provide useful estimates. Based on preliminary analysis of the data, it appears that sweepnet sampling is the most useful method for sampling larval coccinellids in wheat. Adult coccinellids are most efficiently sampled by visual counting. Because larval coccinellids are not always sampled adequately by visual counting, the sweepnet may be the best method for sampling adults and larvae simultaneously.

As part of a continuing effort to develop a comprehensive management system for all economically important aphid pests of small grains in the Great Plains region, we measured the effect of greenbug infestations on yield components of early- and late-planted spring wheat in each of two years. Resulting data were used to develop a model relating yield to cumulative greenbug feeding days (aphid-days). A multiple linear regression model that included different intercepts for each spring wheat planting and a common slope relating yield to aphid-days provided a good fit to the data. Based on the model, a loss of 41 kg of grain per hectare is expected for each 100 aphid-days that accumulate per tiller.

Results of a two-year study of the field population dynamics of the RWA in western Nebraska are currently being analyzed. The data will be used to validate a simulation model of RWA population dynamics. The RWA population model will eventually be expanded into a spatial population dynamics model for use in exploring the effects of landscape structure on RWA population dynamics and management.

Publications Since Last Report

Campbell, R.K., D.K. Reed, J.D. Burd, and R.D. Eikenbary. 1992. Russian wheat aphid and drought stress in wheat: Tritrophic interactions with plant resistance and a parasitoid, pp. 224-234. *In* W.P. Morrison (comp.) Proceedings of the Fifth Russian Wheat Aphid Conference. Great Plains Agric. Counc. Pub. 142.

Campbell, R.K., D.K. Reed, J.D. Burd, and R.D. Eikenbary. 1992. Russian wheat aphid and drought stresses in wheat: Tritrophic interactions with *Diaeretiella rapae* and plant resistance, pp. 297-298. *In* S.B.J. Menken, J.H. Visser, and P. Harrewijn (eds.) Proc. 8th Int. Symp. Insect-Plant Relationships. Kluwer Acad. Publ.

Elliott, N.C. 1991. Biological control of the Russian wheat aphid. *In* Focus on the Russian wheat aphid. Proc. Wheat Technol. Conf., 27-28 February 1991, Chappell/Kimball, Nebraska, Univ. of Nebr., pp. 3-5.

Elliott, N.C. 1992. Simulation modeling, p. 21. *In* R.L. Burton (comp.) The Russian wheat aphid, fourth annual report. U.S. Dep. Agric., Agric. Res. Serv., PSWCL Prog. Rep. 92-001.

Elliott, N.C., D.K. Reed, J.R. Nechols, R.W. Kieckhefer, S.D. Kindler, R.V. Flanders, B.W. French, and D.C. Arnold. 1992. Evaluating Russian wheat aphid parasitoids for establishment potential in the Great Plains, pp. 160-163. *In* W.P. Morrison (comp.) Proceedings of the Fifth Russian Wheat Aphid Conference. Great Plains Agric. Counc. Pub. 142.

Reed, D.K., and N.C. Elliott. 1992. Biological control: Plant Science Research Laboratory, pp. 22-23. *In* R.L. Burton (comp.) The Russian wheat aphid, fourth annual report. U.S. Dep. Agric., Agric. Res. Serv., PSWCL Prog. Rep. 92-001.

Reed, D.K., N.C Elliott, R.V. Flanders, G.L. Hein, M.A. Karner, G.J. Michels, Jr., and C.B. Walker. 1992. Caged versus uncaged releases of Russian wheat aphid natural enemies in four states in spring, 1991, pp. 164-169. *In* W.P. Morrison (comp.) Proceedings of the Fifth Russian Wheat Aphid Conference. Great Plains Agric. Counc. Pub. 142.

Reed, D.K., S.D. Kindler, and T.L. Springer. 1992. Interactions of Russian wheat aphid, a hymenopterous parasitoid and resistant and susceptible slender wheatgrass. Entomol. Exp. Appl. 64:239-246.

Reed, H.C., D.K. Reed, and N.C. Elliott. 1992. Comparative life table statistics of *Diaeretiella rapae* and *Aphidius matricariae* (Hymenoptera: Aphidiidae) on the Russian wheat aphid (Homoptera: Aphididae) in the laboratory, p. 189. *In* W.P. Morrison (comp.) Proceedings of the Fifth Russian Wheat Aphid Conference. Great Plains Agric. Counc. Pub. 142.

Reed, H.C., D.K. Reed, and N.C. Elliott. 1992. Comparative life table statistics of *Diaeretiella rapae* and *Aphidius matricariae* in the Russian wheat aphid. Southwest. Entomol. 17(4):307-312.

Unit Mission: To import, quarantine, test, ship, release, and establish exotic natural enemies (parasites and predators) for use in classical biological control programs for the RWA.

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1992 Quarantine Activities - *Diuraphis noxia* (Mordvilko)
A total of 52 consignments of potential natural enemies of RWA were received at BIIR in 1992. Of the total 11,101 specimens shipped, 10,480 were received alive. The material was collected in Greece, France, Spain, Morocco, Russia, and People's Republic of China. Five of the consignments were coccinellids (only *Hippodamia variegata* and *Coccinella septempunctata*). Two consignments of the syrphid predator *Sphaerophoria* sp., collected in Greece and France, were among material received. Eight consignments of *Leucopis* sp. (possibly *ninae* or *atritarsis*, but no one has yet identified it to species) were received from Morocco and P.R. China and transhipped to APHIS, Niles, MI, for further studies. A subsample of these was also sent to Washington State Univ., Pullman. Fifteen consignments of Braconid parasites were processed from Spain, France, Russia, Morocco, and P.R. China.

Sixty-five shipments (9,149 specimens) of beneficial parasites and predators comprising eight different species were released from quarantine. Predators were sent to R.V. Flanders/D. Prokrym, APHIS, Niles, MI, for evaluation, while the *Coccinella septempunctata* were sent to J.J Obrycki, Iowa State Univ., Ames. Parasite material was shipped to D.K. Reed, ARS, Stillwater; T. Miller/W. Turner, Washington State Univ., Pullman; J.E. Nechols, Kansas State Univ., Manhattan; L.E. Wendel, APHIS, Mission, TX; and D. Gonzalez, Univ. of California, Riverside.

At year end, BIIR continues to maintain (on *Diuraphis noxia*) *Hippodamia variegata* and *Aphelinus asychis* from P.R. China and (on *Acrythosiphon pisum*) cultures of *Adalia bipunctata* (L.), *Coccinella septempunctata* (L.), *Hippodamia variegata* (Goeze), *Propylea quatuordecimpunctata* (L.), and *Semiadalia undecimnotata* (Schneider).

As a result of these activities, this year cooperators made releases (either direct, field cage, or greenhouse cage releases) of *Aphelinus asychis, A. varipes, Aphidius* sp., and *Leucopis* sp. in Washington and *A. asychis* and *A. varipes* in Kansas.

Publications Since Last Report:

Ertle, L.R., and P.W. Schaefer. 1992. Biological control: Beneficial Insects Introduction Research Laboratory, p. 24. *In* R.L. Burton (comp.) The Russian wheat aphid, fourth annual report. U.S. Dep. Agric., Agric. Res. Serv., PSWCL Prog. Rep. 92-001.

Unit Mission: To develop fungal pathogens as antagonists for the RWA in the field, along with appropriate strategies for introduction, and to provide taxonomic support to scientists researching such pathogens.

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Steve P. Wraight, Research Entomologist
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With the quarantine facility at Ithaca still not certified for RWA, the 1992 research was again conducted almost exclusively in the field and, for the second year, at the University of Idaho's Southwestern Idaho Research and Development Center at Parma, in cooperation with S.E. Halbert and T.M. Mowry. Studies focused on development of methods for introduction of entomophthoralean fungal pathogens into spring populations of *Diuraphis noxia*, with the objective of inducing disease epizootics earlier in the season than they naturally occur (usually in July).

Two different methods of introduction were investigated. In the first, adults of the aphid parasitoid *Aphelinus asychis* were heavily inoculated with conidia of *Pandora neoaphidis* or *Zoophthora radicans* and released into the field. Aphid populations were then monitored to determine if the wasps transmitted the fungus to the aphids during their host-seeking and parasitizing activities. The second method involved placing small fragments of sporulating fungus directly into rolled wheat leaves harboring large colonies of *D. noxia*.

RWA populations were extremely low following the 1992 winter season in Idaho, necessitating artificial infestation of the field site with laboratory-reared aphids. An infestation level of 20-30% was established by early May in two subplots in an experimental field of spring-planted wheat, and inoculated parasites were released after sunset on 12 May. One thousand wasps inoculated with *P. neoaphidis* were released into one subplot, and 1000 wasps inoculated with *Z. radicans* were released into the other subplot. Subsequent sampling of aphid-infested tillers from both subplots on 18 May revealed high levels of parasitism (>35% of the late-instar and adult *D. noxia* population); however, no aphids were found infected with either fungus. Second-generation wasps (eggs and first-instar larvae) were not detected until 12 June, and the level of parasitism of the aphid population outside the release subplots remained extremely low (<1%) until the end of the study.

The test of the second method of fungus introduction was initiated on the evening of 29 May in two subplots established in areas of infestation utilized in the parasite release study. In one subplot, small pieces of sporulating mycelium of *P. neoaphidis* were placed directly into approximately 200 rolled wheat leaves with large colonies of RWA. The process was repeated with *Z. radicans* in the other subplot. The field was irrigated prior to application of the fungus to create moist conditions.

Initial samples of 10 inoculated tillers were collected 5 days after application (3 June). Most of the mycelium pieces had sporulated intensively, and 17.7% infection was observed among the *P. neoaphidis*-treated aphids and 5.1% in the *Z. radicans*-treated aphids. While *P. neoaphidis* produced large number of primary infectious conidia on dead hosts, *Z. radicans* produced only resting spores, and by 8 June, *P. neoaphidis* infection had increased slightly to 18.2%, while the *Z. radicans* infection rate declined to zero. Sampling within the *P. neoaphidis* subplot on 9 June revealed that the fungus had spread from the inoculated tillers into neighboring untreated tillers, where the infection level was 11%. Infection of aphids in the untreated tillers increased to 17% by 13 June and finally to 44% by 18 June. Although the fungus spread rapidly from the inoculated tillers to the untreated tillers in the subplot, dissemination into the surrounding area of infestation was extremely slow. Infection rates in samples collected 2 meters in any direction from the subplot on 15 June ranged from 1.9 to 8.1%, and at 4 meters 0.0 to 4.5%. In addition, no infected aphids were detected in tillers collected randomly from the field-wide area of infestation on 10 June.

The grain was beginning to mature by mid-June. Infestation was virtually 100%, and the aphid population was rapidly increasing. By 17 June, the population density was approximately 60 aphids per tiller in the north area of the field and 200 per tiller in the south area (a significant difference apparently attributable to lower stand density in the southern half of the field). A random sample of the aphids collected at this time showed 5% infection. Several isolated pockets of intense *P. neoaphidis* activity were discovered in the southern area of the field a few days later, indicating that a field-wide epizootic was finally beginning to develop in the high-density aphid populations. We intensively monitored such late-season epizootics during the 1990 and 1991 field seasons, and it was not an objective to repeat that effort in 1992. The experiment was terminated on 21 June. Conclusions regarding the success or failure of attempts to establish or augment natural fungal epizootics are, with few exceptions, difficult to draw because of the large number of interacting biotic and abiotic variables comprising the field environment. Studies of other entomophthoralean pathogens, however, have indicated that high levels of moisture (near saturation) and temperatures greater than 10 °C are required for a minimum of 10 hours each day to support fungal infection. Thus, while the failure of

Aphelinus asychis to transmit the fungus may relate to a very low mechanical vector capacity (possibly due to such factors as rapid mortality of the spores on the wasp cuticle or removal of the spores through self grooming), an alternative explanation is that environmental conditions were not suitable for infection.

The successful establishment of *P. neoaphidis* in the tillers inoculated with sporulating mycelium occurred during weather conditions more favorable to fungal infection. At the time of fungus application in our trial, many of the aphid colonies were maturing, and we observed aphids walking on the ground in search of healthy tillers. These dispersing aphids probably account for the rapid dissemination of the pathogen within the treatment subplot. Alates were also present in the population, and may have carried the fungus to more distant points in the field, but apparently only at a slow rate. In addition, field-wide epizootic development was almost certainly inhibited by unstable weather conditons beginning on the fourth night post-application. Conditions suitable for fungal infection returned only after mid-June.

Slow spread of entomophthoralean fungi into the field-wide aphid population from experimentally established infection foci has been reported by a number of investigators, and although a few detailed epizootiological studies have been conducted, the factors determining rates of disease spread remain poorly understood. The spectacular late-season fungal epizootics commonly observed to decimate cereal aphid populations worldwide are often attributed to the biotic factors of high host density and declining vigor in the aging host population. However, in the western United States, RWA populations typically reach high levels by late spring, but natural fungal epizootics rarely develop before early to mid-July. This fact, combined with the observations of this study, suggest that unstable (windy and cold) weather may be a primary factor inhibiting fungal disease outbreak and spread during the spring season. Such a conclusion has important implications and will greatly impact our future research effort. Specifically, in light of these findings, our 1992 proposal to develop a control strategy based on artificial induction of early season epizootics must be reevaluated.

At present the greatest benefit to be gained from initiation or augmentation of entomophthoralean fungal epizootics may be that derived from reduction of the oversummering and overwintering aphid populations. Such benefits are generally considered minimal because of the explosive potential of aphid populations to increase under favorable environmental conditions. However, any factor significantly reducing overwintering populations of *D. noxia* could be useful if the ultimate result were more efficient control of the early spring population by the complex of exotic and native parasites and predators currently being introduced and studied. Our 1991

field studies, for example, indicated that the extremely small aphid population that survived the winter of 1990 on fall-planted grain near Parma, ID, was effectively targeted by the aphidiid parasitoid *Diaeretiella rapae*, with the rate of parasitism exceeding 75% by early June. Additionally, this approach to aphid control may be highly compatible with the long-term goals of plant breeders to develop *D. noxia*-tolerant cereal cultivars. Fungal epizootics could then be manipulated to control aphid populations reaching the economic threshold during late spring or early summer. We have, in fact, documented the potential of *P. neoaphidis* to reduce populations of other important cereal aphids such as the greenbug and the English grain aphid, whose populations, under normal conditions, do not reach economic levels until late spring or early summer.

During early 1992 in Ithaca, we initiated studies exploring the possibility of selecting for enhanced low-temperature activity of *P. neoaphidis* and *Z. radicans*. Lack of an insect quarantine facility greatly hindered this work. The exclusive production of resting spores by the strain of *Z. radicans* released in the above-described experiment was likely induced by low temperature conditions. This problem might have been anticipated had laboratory bioassays been conducted prior to the field season. These results demonstrate the importance of continuing this line of research in the now completed, certified quarantine facility.

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Unit Mission: To collect, study, and import natural enemies of the RWA.

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Exploration/Collection for Natural Enemies
We made trips in China, Egypt, France, Morocco, Nepal, Pakistan, Russia, and Spain that yielded parasitoids, pathogens, and predators of the RWA, *Diuraphis noxia* (Kurdjumov) (Table 1). We collected over 7,964 natural enemies in 18 taxa.

Table 1. Natural enemies of *Diuraphis noxia* collected during exploration by the EBCL in 1992.

Taxon	Countries
Parasitoids:	
Aphelinus asychis Walker	China, France, Morocco
Aphelinus varipes (Foerster)	China, France, Russia
Aphelinus sp.	China
Aphidius spp.	China, Spain, Morocco, Russia
Diaeretiella rapae (McIntosh)	China, Spain, Morocco, Russia
Ephedrus sp.	France
Praon sp.	France, China
Predators:	
Coccinella septempunctata L.	China, France
Hippodamia sp.	China
Leucopis spp.	China, France, Morocco
Episyrphus balteatus De Geer	France
Metasyrphus corollae Fabricius	France
Sphaerophoria scripta L.	France
Pathogens:	
Zoophthora radicans (Bref.) Bat.	France
Beauvaria bassiana (Bal.) Vuill.	France
unidentified spp.	Russia
Parasites:	
mites (to be identified)	Spain, China

Collections of pathogens were made in a broad range of climatic and environmental conditions in Europe, the Middle East, and southern Asia. A total of 82 isolates of Hyphomycete and entomophthoralian fungal pathogens were made from cereal aphids in five countries (France = 21 isolates; Russia = 25 isolates, north of Caucasus; Egypt = 4 isolates, delta north of Cairo; Pakistan = 26 isolates, Lahore and Multan; Nepal = 6 isolates, Kathmandu Valley) and stored in liquid nitrogen at EBCL.

Shipment of Natural Enemies to Cooperators

Over 13,728 predators and parasitoids from 20 cultures from seven countries (Table 2) were shipped to U.S. quarantines (BIIR, Newark, DE, and the Entomology Department, Texas A&M Univ., College Station) and on to cooperators for research and release: D. Prokrym, APHIS, Niles, MI; L.E. Wendel, APHIS, Mission, TX; D.K. Reed, ARS, Stillwater; J.J. Obrycki, Iowa State Univ., Ames; J.E. Nechols, Kansas State Univ., Manhattan; F. Gilstrap, Texas A&M Univ., College Station; D. Gonzales, Univ. of California, Riverside; W. Turner/T. Miller, Washington State Univ., Pullman, D. Yu, Agriculture Canada. Pathogen isolates collected in Pakistan, Nepal, and near Montpellier were shipped to the ARS fungal collection at PPRU, Ithaca, NY. Selected isolates were also shipped to B. Papirok, Pasteur Institute, Paris, for more detailed taxonomic studies.

Dynamics of *D. noxia* and its Natural Enemies

We sampled for *D. noxia* in 112 wheat and barley fields in the Montpellier region. Twenty-seven wheat and 15 barley fields had populations of *D. noxia* (i.e., 38% of the fields were infested). Aphid densities peaked at 70-500/m^2 in spring, and percent infested stems ranged from 2 to 5%, rather independently of aphid density. We found alate aphids all year, but we have yet to find sexual forms. Predator densities varied from 0 to 7/m^2, the coccinellid *Coccinella septempunctata* and syrphids *Episyrphus balteatus, Metasyrphus corollae*, and *Sphaerophoria scripta* being by far the most abundant. Mummified aphid densities varied from 0 to 50/m^2, *Aphelinus varipes* and *A. asychis* being the most abundant.

We placed potted plants, artificially infested with *D. noxia*, in the field monthly from March through December in two experimental wheat fields separated by 4 km. Half the plants were exposed to natural enemies and half were enclosed in cages. We harvested the plants after 2 weeks and recorded the numbers of aphids and mummies (both present at collection and formed later). We also artificially infested the wheat fields with *D. noxia* and sampled parasitoids on tillers with symptoms from March through June. Peak levels of parasitism on the uncaged potted plants were high: 75% on the potted plants in one field in June and 24% in the other in May. The mean density of aphids on uncaged pots (170/plant) was over tenfold lower than on caged plants (2,881/plant). This reduction resulted from the combined

Table 2. Shipments of *Diuraphis noxia* natural enemies made by the EBCL in 1992.

Taxon	Source	Number
Aphelinus asychis	China	1,004
Aphelinus asychis	Kazakstan	1,163
Aphelinu asychis	Morocco	1,560
Aphelinus varipes	China	4,605
Aphelinus varipes	Kazakstan	280
Aphelinus varipes	Russia	865
Aphidius sp.	France	200
Coccinella septempunctata	China	152
Coccinella septempunctata	France	70
Coccinella septempunctata	Russia	50
Diaeretiella rapae	China	340
Diaeretiella rapae	Morocco	654
Diaeretiella rapae	Russia	387
Diaeretiella rapae	Spain	410
Hippodamia sp.	China	282
Leucopis spp.	China	570
Leucopis spp.	France	103
Leucopis spp.	Morocco	700
Sphaerophoria scripta	France	93
Syrphidae	Greece	240
	TOTAL	13,728

action of parasitoids and predators and indicates the important role of natural enemies in limiting *D. noxia* population growth throughout the year.

Five to six species of parsitoids in two familes (Aphelinidae and Aphidiidae) were found. Present in the field throughout the collection period, *Aphelinus asychis* (65% of those collected and *Aphidius* spp. (21%) were the most abundant and second most abundant parasitoids collected. *Aphelinus varipes* (8%) and *Diaeretiella rapae* (4%) were next in abundance and were present in May-July and May-June, respectively. *Praon* sp. (1%) and *Ephedrus* sp. (<1%) were very rare and only found in late fall.

We sampled syrphid pupae and coccinellid larvae and pupae in the experimental wheat field at La Valette using random 1-m^2 quadrats from May to July. We found *S. scripta* was the most abundant syrphid (although parasitism was high [70%]) and *C. septempunctata* was the most abundant coccinellid.

For the third year, we repeated a field exclosure experiment designed to measure the impact of natural enemies on *D. noxia* in the Montpellier area. We treated wheat plants with insecticide to remove resident insects. and artificially infested patches of 10-15 tillers with *D. noxia* to reduce variance between replicates. We then exposed each infested patch to one of three

treatments: 1) closed cages that completely protected the aphids from natural enemies, 2) open cages that allowed access by natural enemies but control for the effect of the cage on microclimate, and 3) no cage so that the local community of natural enemies had access to the aphids. The density of aphids was signficantly higher on plants where natural enemies were excluded than on plants where natural enemies were allowed access. The difference in aphid density was tenfold at 7 weeks after infestation.

Biology of *D. noxia* Natural Enemies

In a field enclosure experiment, larvae of *Sphaerophoria scripta* and *Leucopis* sp. and adult *Aphelinus asychis* were introduced at various densities into cages with wheat plants that had been artificially infested with *D. noxia*. *Sphaerophoria scripta* larvae significantly reduced the density of *D. noxia* compared with cages without syrphids. One larva per cage reduced density from 254 to 69 aphids per cage. On the other hand, the presence of *Leucopis* sp. and *A. asychis* has no significant impact on *D. noxia* density.

A field experiment on the interaction between *Zoophthora radicans* and *Aphelinus asychis* and their effect on *D. noxia* was conducted in 1-m^3 cages in an experimental wheat field near Montpellier in spring. Incidence of fungal infections was greater in plots with both agents than with *Z. radicans* alone. Numbers of *D. noxia* were also lower in plots with both agents.

In a no-choice field cage experiment, we exposed individual *S. scripta, M. corollae*, and *E. balteatus* females to pots with the following *D. noxia* densities: no aphids, low density (20-30 aphids/pot), and high density(350-450 aphids/pot). Oviposition increased with *D. noxia* density. However, the number of eggs laid and the strength of response to aphid density varied with syrphid species.

To test *S. scripta* preferences for aphid species, adult females were released in field cages with brome grass artificially infested with *Sitobion avenae, Rhopalosiphum padi*, and *D noxia*. Low temperature and rain caused losses of all species of aphids, and particularly *S. avenae*. Nonetheless, the density of *D. noxia* was thirtyfold lower in cages with syrphids than in cages without syrphids. However, there was no difference in density of *R. padi* in the presence versus the absence of *S. scripta*.

An isolate of *Beauveria bassiana* collected near Montpellier demonstrated very good potential in laboratory assays for control of *D. noxia*. Isolates of *Paecilomyces fumosoroseus* collected from *Bemisia tabaci* in western Asia also demonstrated promise in laboratory assays as a microbial control agent of *D. noxia*.

To measure the effect of aphid density on oviposition by

Leucopis sp., we exposed adult female flies to wheat plants infested with five densities of second-third instar *D. noxia* ranging from 0 to 100 aphids/cage. The number of eggs laid increased significantly with aphid density from no eggs with no aphids to 20 eggs with 100 aphids per cage. To test the effect of type of leaf surface on oviposition, we exposed female flies to flat versus rolled leaves infested with aphids. The females laid three times more eggs on rolled leaves than on flat leaves.

In a laboratory experiment, we found that lack of aphids for host-feeding severely reduced the egg load of *A. asychis*. The mean number of mature eggs in *A. asychis* females dropped from 10 to 2 within 4 days of being deprived of aphids for host feeding, whereas the mean number of females provided with aphids did not decline, despite oviposition. At the same time, the mean number of resorbed eggs detectable in the ovaries of aphid-deprived females increased from 0 to 10, whereas the mean number of aphid-provided females remained at zero.

Aphelinus asychis females deprived of aphids (but fed honey) for 10 days and *A. asychis* females provided aphids for 4 days fed on the same number of aphids when exposed to aphids for 4 hours, but aphid-provided females parasitized twice as many hosts as aphid-deprived females.

In laboratory experiments and field releases, *A. asychis* males did not respond to odors from females, from aphids, or from host plants. However, males did respond to a contact pheromone left on the substrate by females and to a contact kairomone produced by aphids. A mathematical model of random search by males for plants marked by females could explain the sex ratio observed in the field, given field densities in the Montpellier area and reasonable assumptions about male movement and giving up time.

In cooperation with R.T. Roush, Cornell Univ., we analyzed published data and developed a mathematical model of the population dynamics of introduced parasitoids to explore the possibility that biological control introductions fail because an Allee effect causes small, introduced populations to go extinct. A manuscript was submitted and accepted on this research.

Using allozymes as genetic markers, we examined the genetic structure of *A. asychis* populations in the Montpellier region. There was significant genetic differentiation within fields between patches of about 40 barley stems infested by *D. noxia*, but there was no genetic differentiation among fields in the Montpellier region. These results suggest that during a foreign exploration/collection trip, a sample of representative genetic variation in *A. asychis* from the Montpellier region could be obtained by sampling a moderate number of patches from a single field. Observed levels of between-patch differentiation indicate that on average three female *A. asychis* visited each

patch. Because each patch contained only about eight progeny, each foundress may contribute only two to three progeny to a patch. This is true despite the large number of suitable aphid hosts present on each patch. It also appears that *A. asychis* may have a localized mating structure; we estimate that 52% of all matings may occur within patches. However, the confidence limits for this percentage are extreme. We attempted similar genetic studies for field populations of *Diaeretiella rapae*. However, for this species the frequency of the available allozymic variants were too skewed (i.e., one very common allele and one to several rare alleles) to be informative.

In cooperation with R.T. Roush, Cornell Univ., and W. Powell, Rothamsted Experimental Station, we reviewed the literature relevant to the management of genetics of biological control introductions. This review was accepted for publication in the Annual Review of Entomology.

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